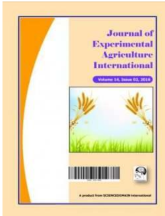

Journal of Experimental Agriculture International
16(1): 1-10, 2017; Article no.JEAI.31780
Previously known as American Journal of Experimental Agriculture
ISSN: 2231-0606

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Determination of Effect of Cold Stratification Temperature and Duration on Germination of Spruce (*Picea smithiana* Wall. Boiss) under Laboratory Conditions

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Authors' contributions

This work was carried out in collaboration between all authors. Authors JAM, PAK and KNK designed the study, performed the statistical analysis and wrote the protocol, while the literature search and drafting of the manuscript was managed and done by authors NAM and GNP. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JEAI/2017/31780
Editor(s):
(1) Mariusz Cycon, Department and Institute of Microbiology and Virology, School of Pharmacy, Division of Laboratoty Medicine, Medical University of Silesia, Poland.
Reviewers:
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(5) Cengiz Yücedağ, Mehmet Akif Ersoy University, Turkey.
Complete Peer review History: http://www.sciencedomain.org/review-history/18755

Original Research Article

Received 24th January 2017
Accepted 6th March 2017
Published 22nd April 2017

ABSTRACT

Aim: The natural regeneration of *Picea smithiana* is generally slow and almost negligible due to a number of factors e.g. presence of undecomposed raw humus on forest floor, low germinative capacity of seed and long interval between good seed years (5-6 years). Therefore the present investigation was carried out to find the effect of stratification temperatures and duration on the germination of *Picea smithiana* under laboratory conditions.
Area of Study: The germination study was carried out in laboratory at Faculty of Forestry,

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Sher-e-Kashmir University of Agriculture Science and Technology of Kashmir, Benhama, Ganderbal, Jammu and Kashmir during 2010-2011 to investigate the effect of different stratification temperature and durations on germination of *Picea smithiana* under laboratory conditions.
Methodology: For germination tests the species seeds were subjected to different stratification temperatures i.e. 0±1℃, 4±1℃, ambient temperature (room temperature) and durations i.e. (15, 30, 45, 60, 75, 90, 105 and 120 days) on germinability of spruce (*Picea smithiana* Wall. Boiss) seeds so that it can be propagated. The experiment was laid out in a completely randomized design with twenty-four treatments combinations replicated three times.
Results: It was observed that stratification temperature 0±1°C (T_1) for a period of 75 days (P_5) resulted in higher germination of 60.62% and germination capacity of 69.62%, which declined to 46.37% and 56.37% at the end of 120 days stratification period. Similar trend was observed for the other germination parameters viz. germination energy, germination value and germination speed.
Conclusion: The germination results indicated that *Picea smithiana* seeds do possess inherent dormancy which increases with storage. Pre-chilling or cold stratification for 75 days at 0±1℃ effectively overcame dormancy in *Picea smithiana*.

Keywords: Picea smithiana; cold stratification; germination; dormancy.

1. INTRODUCTION

Conifer forests are important source of industrial wood and are open treasures, a rich heritage of temperate and subtropical regions of world [1]. Besides providing large quantities of timber, fuel wood and fodder, they are the basic source for procurement of non-wood forest resources and mineral products and are also useful in preservation of natural animal wealth and sustainable ecosystems [2]. In India, conifers occupy extensive forest areas in the Western Himalayan states of Himachal Pradesh, Jammu & Kashmir and Uttrakhand [1]. They play a vital role in maintaining the perennial water system, oxygen flow, conserving and protecting the soil resources, enhancing the beauty of landscape and assuring a soluberent climate. The Himalayas are the home of conifers and constitute one of the most divergent and economically important group of species. The conifer species especially Kail (*Pinus wallichiana* A.B.Jacks.), Deodar (*Cedrus deodara* D. Don), Fir (*Abies pindrow* Royle ex. D.Don) and Spruce (*Picea smithiana* Wall. Boiss) are distributed on an altitudinal line one above the other in tiers, in pure or in mixed species combination. These conifer species form extensive forests of economic value in the Himalayas. *Picea smithiana* is among one of the important conifers of J&K [3]. This conifer species occurs throughout the Western Himalayas from Afghanistan to Kumaun, chiefly at an elevation of 2,000 to 3,200 m above sea level, though occasionally descending lower on northern aspects. Spruce is a tall evergreen tree and due to its gregarious nature, it is not often found pure over large area but more commonly associated with fir or with deodar, blue pine, oak (*Quercus dilatata* Lindl. ex A. DC) and occasionally at higher elevations with *Q. semicarpifolia* (Sm) and other level species such as maple (*Acer ceasium* Wall ex Brandis), walnut (*Juglans regia* L.), bird cherry (*Prunus padus* L.) and elm (*Ulmus wallichiana* Planchon) [2].

Conifer seeds in general have a high degree of dormancy even if subjected to environmental conditions favourable for germination [4]. This dormancy is caused by a combination of internal (physiological) and external (physical) factors [5]. Hard seed coat acts as a barrier for the imbibition of water and exchange of gases, essential for initiation of the germination process. Hard seed coats together with pericarps and other structural barriers impose a high mechanical resistance and block water uptake and/or oxygen diffusion [6]. Cold stratification has been widely used as a pre-sowing treatment for breaking dormancy to enhancing the seed germination rate [7,8]. This is an effortless, cheap and successful method for overcoming seed dormancy. The effects of moist chilling in establishing hormonal levels have been proved due to initiation of appropriate enzyme activity [9]. Moreover, the phenomenon of cold stratification has long been recognized in overcoming physiological dormancy of seeds of many species [10]. Moist chilling breaks the dormancy and accelerates the rate of germination in physiologically dormant *Picea glauca* seeds [11]. Moist chilling of dormant seeds may generally be efficacious, particularly if damage has accumulated due to natural deterioration or as a result of an imposed accelerated ageing regime [12].

The natural regeneration of spruce (*Picea smithiana*) is generally poor because of low germination capacity of seed and infrequent seed formation years. In view of difficulties in getting natural regeneration and the role of chilling duration and incubation temperature in enhancing the germination and other germination related parameters, the present study was under taken to investigate the effect of different stratification temperature and durations on germination of *Picea smithiana* under laboratory conditions.

2. MATERIALS AND METHODS

2.1 Seed Source

Seeds were obtained from mature cones of randomly selected healthy and mature trees from the forest area of Anantnag, Jammu and Kashmir, India in 2010. The forest area is around 2200 m above sea level. Several hundred cones were and packed in gunny bags for their transportation to the experimental site. The cones were dried under shade at room temperature and the seeds were extracted from the cones. The bulk seeds, thus collected, were used for research studies. The germination study was conducted in laboratory at Faculty of Forestry, Sher-e-Kashmir University of Agriculture Science and Technology of Kashmir, Benhama, Ganderbal, Jammu and Kashmir during 2011-2012.

2.2 Stratification Temperatures

During January 2011 and 2012 the collected seeds of spruce were subjected to cold stratification at three different temperatures kept in poly bags in refrigerator.

- T_1 : Dry cold stratification at 0±1℃
- T_2 : Dry cold stratification at 4±1℃
- T_3 : No stratification (at ambient temperature-control)

2.3 Stratification Durations

During each year, the seeds of spruce placed for stratification at different temperatures were drawn separately for each treatment at fifteen days interval (300 seeds /treatment). Seeds drawn from the lot placed at ambient temperature worked as a control for comparison. Seeds were afterwards put to germination test.

Duration		Days	Duration		Days
P1	:	15 days	P5	:	75 days
P2	:	30 days	P6	:	90 days
P3	:	45 days	P7	:	105 days
P4	:	60 days	P8	:	120 days

The experiment comprised of 24 treatments combinations (100 seeds/replication) in completely randomized design under laboratory conditions. After counting, seeds were placed in a petri dish with two fold germination paper and placed in a germinator with a calibrated temperature of 20±1℃.

All treatments were examined daily, seeds were considered germinated when the radicle was 5 mm long.

Germination percentage and germination capacity was recorded daily following formulas given by [13] as bellow:

$$\text{Germination percentage (GP)} = \left(\frac{n}{N}\right) \times 100$$

$$\text{Germination capacity (GC)} = \left[\frac{(v+n)}{N}\right] \times 100$$

where, n is the number of germinated seeds, N is the total number of seeds, v is the number of viable seeds recorded after conducting viability test using tetrazolium chloride [14] and D is the number of days to final germination.

Germination energy, germination speed and germination value was determined using the following formula given by Czabator [15]:

$$\text{Germination energy (GE)} = \left(\frac{M}{N}\right) \times 100$$

Where, M is cumulative germination up to time of maximum MDG reached at any time during the period of the test, N is the total number of seeds, N is the total number of seeds,

$$\text{Germination speed (GS)} = \sum\left(\frac{n}{d}\right)$$

Where, n = number of germinated seeds, d = number of days.

$$\text{Germination value (GV)} = PV \times MDG$$

Where, PV is the peak value of maximum means daily germination reached at any time during the period of the test.

The statistical analysis of each parameter was carried out on mean values and the analysis of variance (ANOVA) was performed using SPSS package (version 12.0). The critical difference (CD) (5 %) was calculated as: CD = SEd X $t_{0.01}$, Where, SEd is the standard error of difference calculated as SEd = $\sqrt{2Me/r}$, where Me= mean sum of square and r= number of replicates.

3. RESULTS AND DISCUSSION

3.1 Stratification of Seed

Seed dormancy is natural way of setting time clock that allows seed to initiate germination when conditions are favourable for germination and seedling survival [11,16]. Embryos described as underdeveloped may be dormant at maturity, thus seeds may need warm and/or cold stratification for embryo growth and germination to occur [5,17]. Various physiological changes occur during the stratification as indicated by increasing or decreasing levels of total sugars and starch in seeds. Combination of low temperature and stratification duration appear to trigger off biochemical changes to transform complex food substances into simple forms that are used at germination time [18]. Cooler temperature during stratification period improves germinability in conifers [19]. The high germinability may be due to conversion of starch into monosaccarides and starch that are used for embryo growth and development [20,18].

3.2 Germination Percentage

The maximum mean germination of 53.81% was recorded in the seeds stratified at 0±1℃ which differed significantly ($p \leq 0.05$) from 50.50 and 46.12 per cent recorded at 4±1℃ and control, respectively (Table 1). A linear increase in the per cent germination was recorded up to 75 days stratification, where after it decreased linearly. Maximum germination of 57.29 per cent was recorded in seeds stratified for 75 days, which was significantly higher than 15 and 30 days period (45.62 and 45.70%, respectively). A linear decrease from the maximum germination was observed after 75 days being 57.20 per cent (90 days), 51.04 per cent (105 days) and 44.37 per cent (120 days), the decrease being significant ($p \leq 0.05$) only in seeds stratified for 105 and 120 days. Interaction between the stratification temperature and period revealed that irrespective of a particular temperature, the maximum germination was observed in the seeds stratified for 75 days. A linear increase in germination was observed as the stratification increased up to 75 days where after a linear decrease was observed. The maximum germination of 60.62 per cent was recorded in the seeds stratified for 75 days at 0±1℃ followed by 60.37 per cent in the seeds stratified for 90 days at 0±1℃ and 59.37 per cent in seeds stratified for 60 days at the same temperature and 75 days at 4±1℃.

3.3 Germination Capacity

Germination capacity measures actual seed germination and potential of non-germinated seeds to germinate after a given time. This gives an index of maturity and viability of seeds. Maximum mean germination capacity of 63.04 per cent was recorded in the seed lot stratified at 0±1℃ which was significantly ($p \leq 0.05$) higher than 61.28 and 56.81 per cent in the seed lots stratified at 4±1℃ and ambient room temperature, respectively (Table 2). The highest mean germination capacity of 67.00 per cent was recorded in the seeds stratified for 75 and 90 days. The effect of interaction of stratification temperature and duration revealed that maximum germination capacity of 69.62 per cent was recorded in seeds stratified for 75 days at 0±1℃, which was closely but non-significantly ($p \leq 0.05$) followed by 69.26 per cent in seeds stratified at the same temperature for 90 days, 69.00 per cent in seed lots stratified for 75 days at 4±1℃ and 68.62 per cent in seed lots stratified for 60 days at 0±1℃.

3.4 Germination Energy

The seed lots scored for germination per cent and germination capacity after stratification for different periods at different temperature regimes were also having maximum germination energy (Table 3). The mean germination energy increased linearly and significantly ($p \leq 0.05$) from 23.29 per cent in the control to 26.63 and 29.18 per cent at 4±1℃ and 0±1℃, respectively. Similarly the maximum mean germination energy (33.46%) was recorded for the seeds stratified for 75 days, which was significantly ($p \leq 0.05$) higher as compared to all other seeds stratified at different intervals stratification. The minimum value of 22.12 per cent was recorded in the seeds stratified for 15 days followed by 22.16 per cent at 120 days, which differed significantly. The interaction between stratification temperature and duration varied significantly ($p \leq 0.05$) with the maximum germination energy of 35.89 per cent recorded for the seeds stratified for 75 days at 0±1℃ followed by the seeds stratified for 75 days at 0±4℃ with 33.65%.

Table 1. Effect of different stratification temperatures, periods and their interaction (T x P) on germination per cent of Spruce (*Picea smithiana* Wall. Boiss) seeds under laboratory conditions pooled over the years 2011 and 2012

Periods / Temperatures	Germination per cent								
	P_1 (15 days)	**P_2 (30 days)**	**P_3 (45 days)**	**P_4 (60 days)**	**P_5 (75 days)**	**P_6 (90 days)**	**P_7 (105 days)**	**P_8 (120 days)**	**Mean**
T_1: 0±1℃	49.12	51.62	55.12	59.37	**60.62**	60.37	47.87	46.37	53.81
T_2: 4±1°C	46.62	47.12	50.62	55.12	59.37	53.87	46.87	44.37	50.50
T_3: Ambient (room temp)	38.37	41.12	42.37	46.62	56.12	53.12	51.37	39.87	46.12
Mean	45.62	45.70	49.37	50.54	57.29	57.20	51.04	44.37	

CD(p ≤ 0.05)

Temperature (T) = 1.68

Period (P) = 2.75

T x P = 4.78

Table 2. Effect of different stratification temperatures, duration and their interaction (T x P) on germination capacity of Spruce (*Picea smithiana* Wall. Boiss) seeds under laboratory conditions pooled over the years 2011 and 2012

Periods / Temperatures	Germination capacity (%)								
	P_1 (15 days)	**P_2 (30 days)**	**P_3 (45 days)**	**P_4 (60 days)**	**P_5 (75 days)**	**P_6 (90 days)**	**P_7 (105 days)**	**P_8 (120 days)**	**Mean**
T_1: 0±1℃	58.25	59.62	60.37	68.62	**69.62**	69.26	57.87	56.37	63.04
T_2: 4±1℃	56.37	56.87	60.37	61.00	69.00	65.25	65.25	6 4.12	61.28
T_3: Ambient (room temp)	47.87	53.16	53.26	56.62	66.12	63.37	56.75	53.87	56.81
Mean	54.79	56.16	59.45	62.41	67.00	67.00	59.58	55.50	

CD (p ≤ 0.05)

Temperature (T) **=** *1.23*

Period (P) **=** *2.01*

T x P **=** *3.48*

Table 3. Effect of different stratification temperatures, duration and their interaction (T x P) on germination energy of Spruce (*Picea smithiana,* Wall. Boiss) seeds under laboratory conditions pooled over the years 2011 and 2012

Periods / Temperatures	Germination energy (%)								
	P_1 (15 days)	**P_2 (30 days)**	**P_3 (45 days)**	**P_4 (60 days)**	**P_5 (75 days)**	**P_6 (90 days)**	**P_7 (105 days)**	**P_8 (120 days)**	**Mean**
T_1: 0±1℃	**19.13** (4.48)	25.21 (5.091)	30.49 (5.59)	32.02 (5.72)	**35.89** (6.07)	33.03 (5.83)	25.07 (5.08)	24.59 (4.95)	29.18 (5.40)
T_2: 4±1℃	20.07 (4.58)	26.06 (5.18)	27.00 (5.27)	29.71 (5.51)	35.65 (5.88)	32.36 (5.77)	21.67 (4.74)	20.26 (4.61)	26.63 (5.22)
T_3: Ambient (room temp)	20.24 (4.56)	21.87 (4.76)	24.38 (5.00)	30.83 (5.63)	31.42 (5.68)	27.83 (5.36)	25.59 (5.14)	20.20 (4.60)	23.29 (4.82)
Mean	22.12 (4.77)	26.97 (5.19)	27.87 (5.27)	28.17 (5.30)	33.46 (5.78)	27.67 (5.33)	24.43 (4.94)	22.16 (4.70)	

CD ($p \leq 0.05$)
Temperature (T) = 0.21
Period (P) = 0.37
T x P = 0.64

Table 4. Effect of different stratification temperatures, duration and their interaction (T x P) on germination speed of Spruce (*Picea smithiana,* Wall. Boiss) seeds under laboratory conditions pooled over the years 2011 and 2012

Periods / Temperatures	Germination speed								
	P_1 (15 days)	**P_2 (30 days)**	**P_3 (45 days)**	**P_4 (60 days)**	**P_5 (75 days)**	**P_6 (90 days)**	**P_7 (105 days)**	**P_8 (120 days)**	**Mean**
T_1: 0±1℃	5.43	6.60	7.61	9.33	**10.92**	9.04	7.97	5.86	9.25
T_2: 4±1℃	6.86	7.88	8.06	8.94	9.53	9.30	7.43	6.78	8.2 3
T_3: Ambient (room temp)	6.82	8.78	9.28	10.37	10.59	10.18	9.34	8.70	7.58
Mean	6.37	8.42	8.68	9.33	9.55	8.81	8.11	7.54	

CD ($p \leq 0.05$)
Temperature (T) = 0.80
Period (P) = 1.31
T x P = 2.28

Table 5. Effect of different stratification temperatures, duration and their interaction (T x P) on germination value of Spruce (*Picea smithiana* Wall. Boiss) seeds under laboratory conditions pooled over the years 2011 and 2012

Periods / Temperatures	Germination value								
	P_1 (15 days)	**P_2 (30 days)**	**P_3 (45 days)**	**P_4 (60 days)**	**P_5 (75 days)**	**P_6 (90 days)**	**P_7 (105 days)**	**P_8 (120 days)**	**Mean**
T_1: 0±1℃	2.49	3.78	4.26	4.89	**4.94**	4.67	4.64	3.78	6.30
T_2: 4±1°C	3.68	3.70	4.06	4.65	4.94	4.44	4.04	4.08	4.20
T_3: Ambient (room temp)	2.54	3.35	3.35	4.67	4.67	4.15	3.46	3.31	3.81
Mean	3.34	3.70	4.01	4.44	4.53	4.46	4.26	3.75	

CD (p ≤ 0.05)

Temperature (T) **=** *0.86*
Period (P) **=** *0.58*
T x P **=** *1.01*

3.5 Germination Speed

Germination speed measures the germination potential of seeds at a particular time scale and gives an indication of the internal physiological factors inside the seed that enhance and promote the germination process when external factors favorable for germination are kept at optimum level. The highest mean germination of 9.25 was recorded for the seeds stratified at 0±1℃, which was significantly ($p \leq 0.05$) higher than 8.23 and 7.58 recorded in the seed lots stratified at 4±1℃ and ambient room temperature, respectively (Table 4). Stratification periods revealed a linear increase in the germination speed up to 75 days interval, where after it decreased linearly. The highest germination value of 9.55 was recorded in the seed lot drawn on 75th day, followed by 9.33 on 60th day.

3.6 Germination Value

The maximum mean germination value of 6.30 was recorded in the seeds stratified at 0 ± 1^0C, which was significantly ($p \leq 0.05$) higher than 4.20 and 3.81 recorded at 4±1℃ and ambient room temperature, respectively (Table 5). The difference between the values 3.81 and 4.20 was non-significant. Maximum mean germination value of 4.53 was recorded in the seeds lot drawn on 75th day of stratification, which was however, non-significantly different from the values of 4.01, 4.26, 4.44 and 4.46 recorded in the seed lots drawn on 45th, 105th, 60th and 90th day, respectively. The maximum value was however, significantly ($p \leq 0.05$) higher than those recorded on 15th day (3.34), 30th day (3.70) and 120th day (3.75). Considering the effect of interaction of stratification temperature and duration, it was observed that maximum germination value of 4.94 was recorded in the seeds stratified for 75th day at 0±1℃ or 4±1℃. Germination values of 3.78 or above at 0±1℃ were significantly higher than that recorded on 15th day (2.49).

Moist stratification at low temperatures or pre-sowing in cold water for a period of few hours to several days is reported to increase germination without influencing germination capacity in seeds of spruce [21]. After 3 weeks of cold chilling of spruce seeds, a significant increase by 56.00 per cent was recorded in germination over control. Germination value and germination speed recorded a significant increase by 29.70 and decrease by 40.0 per cent, respectively in spruce seeds after 4 weeks of moist chilling [22]. Jinks and Jones [23] reported increase in germination speed in *Picea sitchensis* after chilling. Lavania et al. [24] observed an increase in germination by 19.75 per cent after 60 days of stratification of spruce seeds as compared to control. Mugloo et al. [25] reported significant increase in seed germination of *Cryptomeria japonica* up to 45 days of stratification where after it decreased significantly. The cold or winter stratification outdoors successfully overcame seed dormancy for commercial propagation of the trees species [26,27].

Thapiliyal and Gupta [28] reported that germination per cent varied from 16 to 69% in of *C. deodara* seeds and Rawat and Bakshi, [29] also documented wide range of variation with respect to stratification temperature and duration in *Pinus wallichiana*. Sofi and Bhardwaj [30] reported significant improvement in seed germinability parameters of *C. deodara* after cold moist stratification at 2-3℃ for 75 days followed by 60 days of pre-chilling. Malik and Shamet [31] reported significant improvement in seed germination of *Pinus gerardiana* after 60 days of stratification at 4±1℃. Faster germinating seeds are reported to produce more uniform and vigorous seedling that promote the seedling growth and development parameters [32]. Stratification is the most common method employed to break the dormancy so as to ensure uniform and quick germination of seeds in many species [33,34]. Gosling [35] postulated that stratification is interpreted as important tool to encourage germinability of dormant seeds that require pretreatment by duration and temperature. It has synchronized germination of most gymnosperm seeds, even those that exhibit no dormancy. Embryo growth occurs at relatively high temperatures (15℃) in simple morphophysiological dormancy; whereas, in complex morpho-physiological dormancy, embryo growth occurs only at low temperatures (0–10℃) [10,36].

4. CONCLUSION

The study confirms that seeds of *Picea smithiana* have linear underdeveloped embryos at maturity, and exhibit intermediate complex morpho-physiological dormancy. Pre-chilling or cold stratification for 75 days at 0±1℃ effectively overcame dormancy, and accelerated seed germination of *Picea smithiana* and could be used for commercial propagation. Embryonic growth and development occurred during chilling at 0±1℃.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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